Aspirin

After more than 200 years, the mechanisms by which this venerable drug and its relatives achieve their wide range of effects have yet to be fully elucidated

by Gerald Weissmann

There is a bark of an English tree, which I have found by experience to be a powerful astringent, and very efficacious in curing aguish and intermitting disorders.

—The Rev. Mr. Edmund Stone of Chipping-Norton in Oxfordshire, in a letter to the Right Honourable George, Earl of Macclesfield, president of the Royal Society, April 25, 1763

What Stone had discovered, although he did not know it, was that salicylates—the general term for derivatives of salicylic acid—reduced the fever and relieved the aches produced by a variety of acute, shiver-provoking illnesses, or agues. The bark of the willow tree (*Salix alba*) is astringent because it contains high levels of salicin, the glycoside of salicylic acid.

Today the salicylate most commonly used is acetylsalicylic acid, better known by its first trade name, "Aspirin." Americans consume 16,000 tons of aspirin tablets a year—80 million pills—and spend about $2 billion a year for nonprescription painkillers, many of which contain aspirin or aspirinlike drugs.

As Stone observed, these compounds exhibit a wide range of effects: At the lowest doses—less than one tablet a day—aspirin can be used to treat and prevent heart attacks and to prevent cerebral thrombosis. Two to six tablets a day (one to three grams) are useful for reducing pain or fever. And much higher doses (four to eight grams a day) reduce the redness and swelling of joints in diseases such as rheumatic fever, gout and rheumatoid arthritis.

Aspirin and the salicylates also have many other biological effects, only some related to their clinical use. Salicylates can dissolve corns on the toes, provoke loss of uric acid from the kidneys and kill bacteria in vitro. Aspirin inhibits the clotting of blood, induces peptic ulcers and promotes fluid retention by the kidneys.

Cell biologists use aspirin and salicylates to inhibit ion transport across cell membranes, to interfere with the activation of white blood cells and to derail the production of the energy-storage compound adenosine triphosphate by isolated mitochondria. Molecular biologists use the compounds to activate genes that code for so-called heat-shock proteins in the lampbrush chromosomes of the fruit fly, *Drosophila*. And botanists use salicylates to induce flowering of such plants as *Impatiens* and the voodoo lily.

The enormous range of effects that aspirin can produce has made it a complex task to pin down the biochemical mechanisms involved. Not until the early 1970s did biologists find a hypothesis to explain the action of aspirin and such related drugs as ibuprofen, indomethacin and piroxicam. That hypothesis was based on the ability of these drugs to block the synthesis of prostaglandins, cellular hormones involved in pain and inflammation.

More recently it has become clear that the prostaglandin hypothesis explains only some of the effects of aspirin and related compounds. Their crucial anti-inflammatory power appears to stem not only from prostaglandin inhibition but also from their ability to disrupt interactions within cell membranes. Recent work in my laboratory, for example, has shown how aspirinlike drugs prevent the activation of cells that mediate the first stages of acute inflammation.

The story of how willow extracts found their way from the agues of Oxfordshire to the bench side of molecular biologists can be told in terms of the four Gs of Paul Ehrlich: *Geduld, Geschick, Geld und Glück* (patience, skill, money and luck). In the case of Reverend Stone, luck seemed to have shown the way.

In about 1757 Stone tasted willow bark (already a well-known folk remedy) and was surprised at its extraordinary bitterness. The resemblance to the taste of Peruvian bark (*Cinchona*)—a rare and expensive remedy for the ague—excited Stone's suspicions. Six years of careful clinical observation culminated in his letter to the Royal Society. Stone offered a skillful rationale for the use of willow bark in febrile disorders, based on the traditional doctrine of signatures, which held that "many natural maladies carry their cures along with them, or their remedies lie not far from their causes." Willows, as do feverish illnesses, abounded in moist shires.

Half a century later, driven by national rivalry, French and German pharmacologists competed to find the active principle of willow bark. By 1828, at the Pharmacologic Institute of Munich, Johann A. Buchner isolated a tiny amount of salicin in the form of bitter-tasting yellow, needlelike crystals. One year later, H. Leroux in Paris improved on the extraction procedure and obtained one ounce of salicin from three pounds of the bark. In 1833 the pharmacist E. Merck of Darmstadt obtained a clean preparation of salicin that was cheaper by half than the impure willow extracts. Not until 1838 did Raffaele Piria of Pisa, working in Paris, give the compound the name by which it is

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known today: *l'acide salicylique*, or salicylic acid.

Other plants were also rich natural sources of salicylates. Meadowsweet (*Spiraea ulmaria*) yielded ample quantities of an ether-soluble oil from which the Swiss chemist Karl Jakob Löwig crystallized "*Spirsäure*" in 1835. In 1839 Dumas demonstrated that Löwig's *Spirsäure* was none other than Piria's *acide salicylique*. Another Gallic pharmacologist, Auguste Cahours, showed in 1844 that oil of wintergreen—a traditional remedy for aguish disorders—contained the methyl ester of salicylic acid.

As was to be the case in much of 19th-century chemistry, French and British scientists were slightly ahead of the Germans in the study of natural products, but Germans held the edge in synthetic know-how. Forced to compete with the French and British dye industries, which supplied the textile mills of Lyons and Macclesfield with pigments imported from overseas colonies, the Germans replied by inventing cheap aniline dyes, creating in their train such giant enterprises as I. G. Farben. By the mid-1870s German synthetic chemistry was preeminent worldwide. Whereas in the 1860s no German dyes were exported, by 1888 they supplied more than 80 percent of the world's needs.

Germans also began to dominate the willow business. In 1860 Hermann Kolbe and his students at Marburg University synthesized salicylic acid and its sodium salt from phenol, carbon dioxide and sodium. In 1874 one of those students, Friedrich von Heyden, established the first large factory devoted to the production of synthetic salicylates in Dresden. Whereas the price of salicylic acid made from salicin was 100 *Thaler* per kilogram in 1870, by 1874 the price of the synthetic product was only 10 *Thaler* per kilogram.

The availability of cheap salicylic acid spread its clinical use far and wide. In 1876 Franz Stricker and Ludwig Riess, writing in the *Berliner Klinische Wochenschrift*, and T. J. MacLagan, writing in the *Lancet*, reported the successful treatment of acute rheumatism—the disease now known as acute rheumatic fever—with salicylates at doses of five to six grams a day. Unfortunately, only the disease's acute symptoms, not its long-term consequences, respond to salicylates. Patients with rheumatic fever mount an inflammatory response against their own joints as if out at any cost to eradicate bacteria lodged there. The most severe and lasting damage is inflicted on the heart; fully one third of the victims suffer scarring of the valves.

The following year, in Paris, Germain Sée introduced salicylates as effective

WILLOW TREE is a source of salicin, a bitter compound whose derivative, acetylsalicylic acid (aspirin), is now the world's most common remedy for pain and fever. Other plants, such as meadowsweet and wintergreen, also contain salicylates.

therapies for gout and chronic polyarthritis. The latter category includes rheumatoid arthritis, an often crippling, inflammatory disorder of the middle years that affects more women than men, and degenerative osteoarthritis, a painful ailment that affects the knees of football players or the toes of young ballet dancers—and various joints of most people over 60.

Aspirin, now the most common salicylate, entered the competition fairly late. Its discovery, in 1898, began with the arthritic parent of an aniline dye chemist at the Bayer division of I. G. Farben. Felix Hofmann's father could not tolerate sodium salicylate because of chronic and acute stomach irritation. (No wonder, six to eight grams of salicylate a day will predictably irritate almost anyone.) Hofmann searched the chemical literature for less acidic derivatives of sodium salicylate and hit on acetylsalicylic acid. It proved more palatable and—Hofmann claimed—more effective at helping his dad. (I have a hunch that Hofmann *père* had osteoarthritis and got away with lower, analgesic doses of acetylsalicylic acid rather than anti-inflammatory quantities of sodium salicylate.)

Bayer called the new drug aspirin, the "a" from *acetyl* and the "spirin" from the German *Spirsäure* (the French root would have yielded *asalicylin*). By 1899 there was no chemical industry on earth that could compete with the German cartels. The Germans had won the aspirin war and could dictate the terms of victory.

Competitors entered the field as markets expanded for drugs that could reduce fever and pain. Some contenders, in fact, had been invented before aspirin but gained acceptance as over-the-counter remedies in Europe and the U.S. only after aspirin's success at the turn of the century. Based on anecdotal accounts from the Alsace that a product formed from aniline treated with vinegar made a useful febrifuge, or fever treatment, Karl Morner synthesized acetanilide in 1889—essentially an aniline version of acetylsalicylic acid.

Acetanilide itself caused bone marrow depression and anemias, and so other derivatives were sought. The most widely accepted aniline-derived product turned out to be phenacetin: the "P" in the APC that since 1939 has been so beloved by U.S. Army physicians, who have given every recruit with a fever a pill compounded of aspirin, phenacetin and caffeine. Both acetanilide and phenacetin are broken down in the body to form *N*-acetyl-*p*-aminophenol, which by various anagrammatic combinations yielded the generic names "acetaminophen" in the U.S. and "paracetamol" in Great Britain, as well as the trade name "Tylenol."

Neither acetanilide nor phenacetin, however, proved as useful as aspirin in treating rheumatic fever or rheumatoid arthritis, and for half a century (between 1900 and 1950) clinicians appreciated that there was something unique about high-dose salicylates. Doses of more than about four grams of aspirin a day did not just relieve fever and pain; they also reduced swelling and diminished the objective signs of inflammation. In addition, they brought under control such laboratory markers of the disease as the erythrocyte sedimentation rate (the rate at which red blood cells fall through plasma) and the levels of C-reactive protein, a substance produced by the liver in response to infection. Although later such drugs as ibuprofen, indomethacin and piroxicam also acted against inflammation, the mechanism by which they did so was unclear.

By the early 1970s no useful hypothesis had yet explained how salicylates exerted their various effects. Renal physiologists found that low doses of salicylates blocked the excretion of uric acid by the kidneys, thus raising levels of this acid in the blood, but, paradoxically, high doses of salicylates promoted renal excretion and so lowered uric acid levels. The latter property explained the utility of salicylates in both acute and chronic gout.

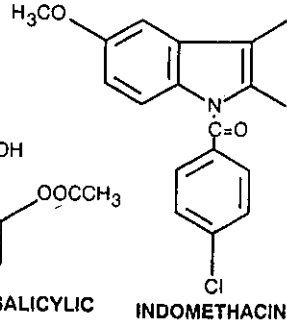
Pharmacologists had shown that salicylates reduce pain by acting on tissues and associated sensory nerves—as opposed to morphine, which acts on the brain. But physiologists main-

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

CARBOXYLIC ACIDS

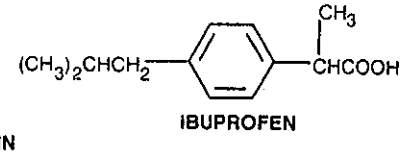
SALICYLATES	*ACETIC ACIDS*	*PROPIONIC ACIDS*	*FENAMATES*
ACETYLSALICYLIC ACID (ASPIRIN)	INDOMETHACIN	IBUPROFEN	MEFENAMIC ACID
SALSALATE	ACEMETACIN	FLURBIPROFEN	FLUFENAMIC ACID
DIFLUNISAL	CINMETACIN	NAPROXEN	MECLOFENAMATE
FENDOSAL	SULINDAC	KETOPROFEN	NIFLUMIC ACID
	TOLMETIN	FENOPROFEN	TOLFENAMIC ACID
	ZOMEPIRAC	BENOXAPROFEN	FLUNIXIN
	DICLOFENAC	INDOPROFEN	CLONIXIN
	FENCLOFENAC	PIRPROFEN	
	ISOXEPAC	CARPROFEN	

PYRAZOLES	OXICAMS
PHENYLBUTAZONE	PIROXICAM
FEPRAZONE	ISOXICAM
APAZONE	TENOXICAM
TRIMETHAZONE	
MOFEBUTAZONE	
KEBUZONE	
SUXIBUZONE	



ACETYLSALICYLIC ACID

INDOMETHACIN



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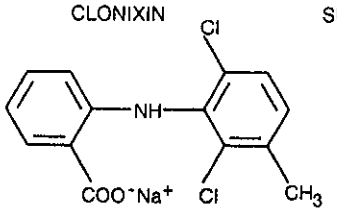
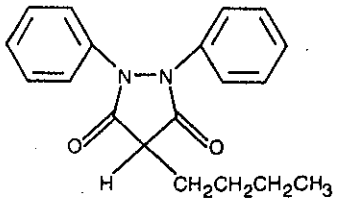
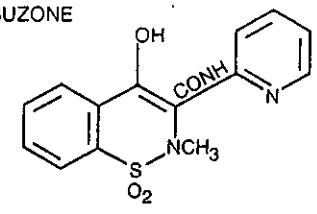


MECLOFENAMATE



PHENYLBUTAZONE



PIROXICAM

tained that salicylates reduced fever by working directly on the fever centers of the hypothalamus, not by peripheral action.

It was even more difficult to explain how aspirin inhibited platelet function, caused salt and water retention and provoked indigestion. And why did some patients develop the nasal polyps accompanied by sniffles and wheezes known as aspirin hypersensitivity?

The first satisfactory mechanism for the action of aspirin was proposed in 1971 by John R. Vane (now Sir John and 1982 Nobelist) and his colleagues at the Royal College of Surgeons in London. That hypothesis moved aspirinlike drugs into the forefront not only of pharmacology but also cell biology and, eventually, clinical medicine.

Vane had been impressed by the fact that many forms of tissue injury are followed by the release of prostaglandins, a group of ubiquitous local hormones produced by the enzymatic oxidation of arachidonic acid, a fatty acid contained in cell membranes. (Prostaglandins have a host of regulatory functions, including regulation of blood vessel tone, uterine contraction and platelet function.) Unlike hormones such as insulin, prostaglandins are not stored within cells but are released when cells are injured or stimulated by other hormones. Moreover, sensitive chemical and biological assays had permitted investigators to show that two particular groups of prostaglandin, E_2 and I_2, caused several of the signs of inflammation, including redness (vasodilation) and heat (fever).

Vane then used radioactively labeled arachidonic acid to demonstrate that aspirin and related drugs inhibited the synthesis of prostaglandins E_2 and $F_{2\alpha}$. Moreover, platelets taken from volunteers given aspirin and indomethacin failed to make prostaglandins in response to the clotting factor thrombin. Finally, indomethacin inhibited the normal release of prostaglandins from dogs' spleens stimulated by the neurotransmitter catecholamine. There was no question that aspirinlike drugs blocked prostaglandin synthesis.

At long last the salicylate story seemed to have found a beginning, middle and end. All that remained, it appeared, was to fill in the details: to show how and when prostaglandins caused redness and swelling with heat and pain and to study the means by which aspirinlike drugs inhibited the enzyme—now known as prostaglandin H synthase—that transformed arachidonic acid to prostaglandins. That enzyme produces stable prostaglandins (those of the E, I and F series) via the unstable intermediates PGG_2 (prostaglandin G_2) and PGH_2 (first discovered in the 1970s by another 1982 laureate, Bengt Samuelsson of the Karolinska Institute in Stockholm).

By 1974 Vane and Sergio Ferreira had amassed convincing evidence for the prostaglandin hypothesis. Almost all aspirinlike drugs (by then generally called nonsteroidal anti-inflammatory drugs, or NSAIDs) inhibited prostaglandin synthase, and the potency of these drugs pretty much paralleled their effectiveness. Aspirin was anywhere from 1/40 to 1/200 as active as indomethacin and from one fifth to 1/50 as active as ibuprofen. Furthermore, such centrally acting analgesics as morphine or codeine did not inhibit prostaglandin synthase; neither did antihistamines, antiserotonin drugs or cortisone and its analogues.

Vane and his colleagues argued not only that prostaglandins were produced at sites of inflammation but also that they could, alone or in concert with other mediators, provoke the cardinal signs of inflammation. Indeed, prostaglandins E_2 and I_2 do induce vasodilation and promote swelling when dilated blood vessels have been made leaky by histamine. They also produce fever when injected either into the cerebral ventricles or into the anterior hypothalamus, and they sensitize pain receptors of the skin to such other pain-provoking substances as bradykinin or histamine.

Perhaps the most persuasive aspect of the prostaglandin hypothesis was its explanation of the clinical side effects of NSAIDs. Their most troublesome side effect is stomach irritation and ulceration—aspirin is the worst offender in this regard. The drugs cause this irritation because they block the synthesis of prostaglandins that the stomach lining needs to regulate overproduction of acid and to synthesize the mucus barrier that prevents its self-digestion.

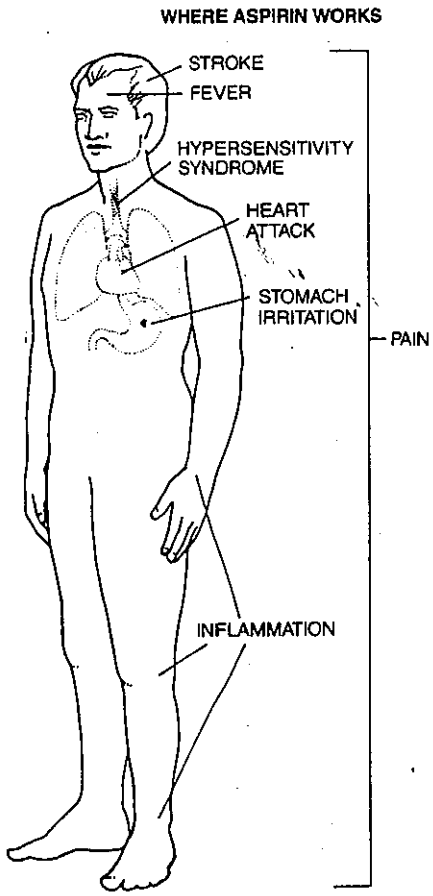
In addition, most NSAIDs prevent the body from excreting salt and water properly, particularly when heart or liver disease compromises blood flow to the kidneys. When NSAIDs prevent the kidneys from synthesizing PGI_2 (a prostaglandin that causes blood vessels to dilate), the renal blood supply is reduced even further; patients sometimes accumulate enough fluid to choke their circulation.

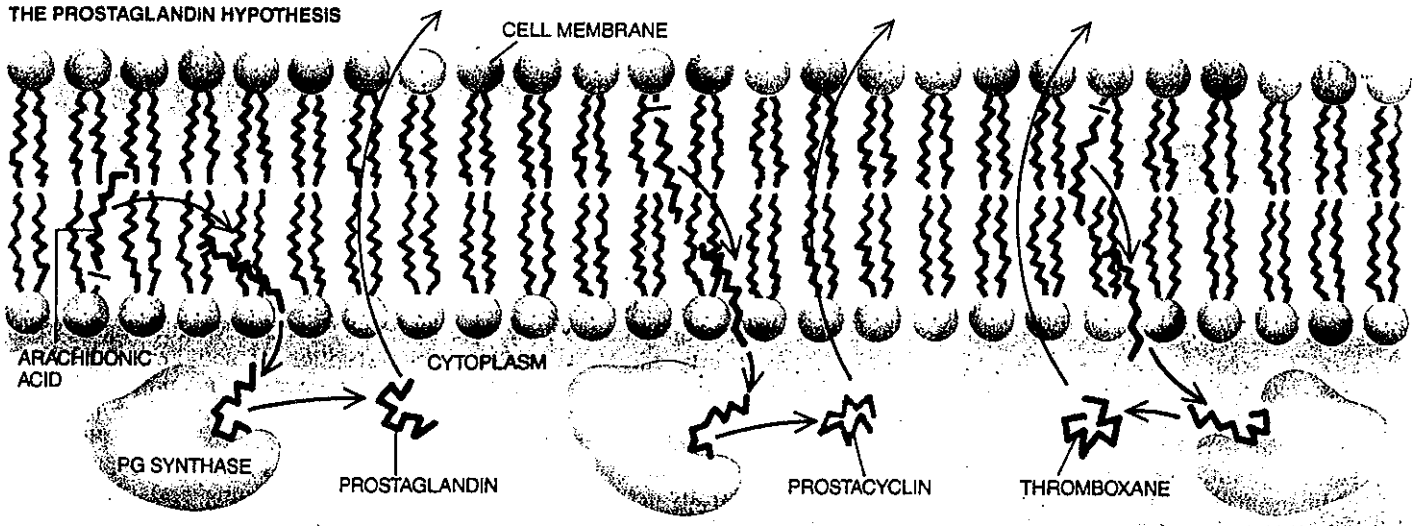
Another side effect of NSAIDs—but not sodium salicylate—is aspirin sensitivity syndrome in genetically susceptible patients. It turns out that blocking prostaglandin synthase diverts arachidonic acid to another pathway that transforms it to a host of substances—notably leukotriene B_4, C_4 and D_4—that exceed in irritative potency the products of PGH synthase.

Finally, the most common side effect of NSAIDs, and especially aspirin, is interference with the clotting of blood. Patients who take these drugs sometimes suffer from untoward bleeding after tooth extraction, minor surgery or trauma. Aspirin inhibits platelet aggregation (the cellular aspect of blood clotting), and other NSAIDs—again with the exception of sodium salicylate—also inhibit platelet function.

NSAIDs act by blocking the production of both PGG_2 and PGH_2. Platelets transform the latter into a most potent vasoconstricting and platelet-aggregating substance, thromboxane B_2. Meanwhile the endothelial cells that line blood vessels use those same prostaglandin intermediates to make a potent vasodilator, prostacyclin, or PGI_2.

These seemingly arcane discoveries form the basis for using aspirin to prevent strokes and heart attacks: carefully calibrated doses can interfere with thromboxane production while leaving prostacyclin synthesis unaffect-





Swelling, heat and pain are caused by prostaglandins that cells synthesize in response to injury. The enzyme PG synthase transforms cell membrane component arachidonic acid into unstable prostaglandin intermediates and then into stable prostaglandins.

Endothelial cells turn prostaglandin intermediates into prostacyclin, a vasodilator, whereas platelets use them to synthesize thromboxane, a potent vasoconstrictor and clotting agent. As a result, selective inhibition of PG synthase in platelets can reduce the risk of heart attack and stroke.

ed. Aspirin irreversibly inactivates PGH synthase. Platelets cannot make more synthase and so make no more thromboxane. Endothelial cells, however, can make new synthase, so prostacyclin synthesis is inhibited only a few days.

Furthermore, Garret A. FitzGerald and John Oates of the Vanderbilt University School of Medicine have shown that less than one tablet a day can irreversibly block the PGH synthase activity of platelets in the portal circulation (the circulation that drains the intestines via the liver), thus reducing the risk of dangerous clots, before appreciable amounts of aspirin appear in the general circulation, where they might disrupt prostacyclin production.

No discovery that has evolved from Vane's hypothesis has had more of an impact on public health: the hundreds of thousands of patients around the world who now take aspirin to treat or prevent strokes and heart attacks owe Sir John a not inconsiderable debt.

The prostaglandin hypothesis certainly explained the effects of very low (antithrombotic) and intermediate (analgesic and antipyretic) doses of aspirin. Although there were some troubling discrepancies, Vane and his colleagues gave sound explanations for them. For example, acetaminophen does not keep prostaglandins from synthesizing, but it is effective against the synthase from the brain (perhaps explaining its antipyretic effect). And although nonacetylated salicylates are roughly one tenth as potent against prostaglandin synthase in vitro as aspirin—indicating that they should be ineffective analgesics—studies of prostaglandin metabolites show that sodium salicylate may indeed inhibit prostaglandin synthesis in the body.

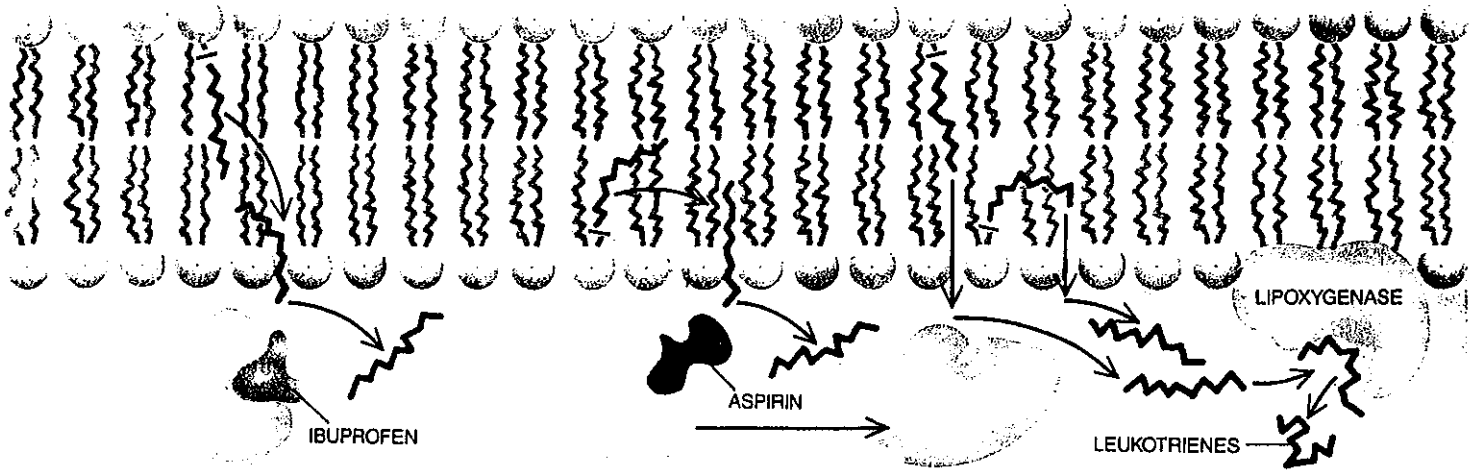
Vane's theory that the local production of prostaglandins leads to inflammation has only partly been substantiated, however. The anti-inflammatory effect of salicylates requires much higher doses than does analgesia. This discrepancy suggests either that the prostaglandin synthase of cells provoking inflammation is relatively insensitive to aspirin or that aspirin owes its anti-inflammatory property at higher concentrations to a mode of action distinct from its ability to inhibit prostaglandin synthesis.

The properties of sodium salicylate and acetaminophen provide further evidence that aspirinlike drugs exert clinical effects that do not depend on inhibiting prostaglandin synthesis. Sodium salicylate shares many of the analgesic properties of aspirin, but it fails to inhibit prostaglandin synthesis in disrupted cell preparations at concentrations that can be achieved in the body. It also does not inhibit platelet function or cause bleeding. And today's most widely used analgesic and antipyretic drug, acetaminophen, does not inhibit prostaglandin synthesis; nor does it keep blood from clotting or reduce inflammation. Pain and fever, it seems, can effectively be reduced without inhibiting the synthesis of prostaglandins at all.

The broad spectrum of NSAID effects most likely results from their physical properties, which permit them to disrupt interactions within biological membranes. NSAIDs are planar, anionic molecules that have an affinity for lipid environments such as the lipid bilayers of plasma membranes. Moreover, the more acidic the environment (as at inflammatory sites), the greater the lipophilicity of NSAIDs. It is therefore not surprising that these drugs interfere with many functions of inflammatory cells.

For example, aspirin alters the uptake of fatty acids and their insertion into the membranes of cultured human monocytes and macrophages. Salicylates also inhibit anion transport across a variety of cell membranes. Finally, NSAIDs inhibit bone metabolism and the synthesis of proteoglycan, a substance that forms the matrix of cartilage, by mechanisms that do not depend on the inhibition of the prostaglandin synthase. The last point does not merely weaken the prostaglandin hypothesis; it is also a matter of significant clinical concern.

Recent work in my laboratory has uncovered an alternative mechanism for the effects of aspirinlike drugs: interference with stimulus-response coupling in neutrophils, the most abundant cells of acute inflammation. These cells are the first line of defense against foreign intruders and among the first to cause injury in autoimmune diseases like rheumatoid arthritis. They damage tissues by releasing enzymes



Aspirin can selectively inhibit PG synthase in platelets because it permanently deactivates the enzyme, whereas some other aspirinlike drugs block the enzyme only temporarily. (Endothelial cells continue producing prostacyclin because they can make new synthase to replace that destroyed by the aspirin.)

Hypersensitivity syndrome afflicts some individuals exposed to aspirin. Once the action of PG synthase is blocked, a competing enzyme, lipoxygenase, transforms arachidonic acid into substances that have even more irritative power than prostaglandins.

that break down proteins (proteases), as well as inflammatory peptides, reactive oxygen species such as O_2^- and H_2O_2 (peroxide), and lipid irritants such as platelet-activating factor and leukotriene B_4.

Within five seconds after coming into contact with substances that provoke inflammation (immune complexes, complement components—a cascade of enzymes and bioactive peptides that interact with antibodies to provoke immune response—and other chemoattractants), the neutrophil is transformed into a secretory cell capable of provoking tissue injury. One of the first steps in tissue injury is neutrophil aggregation, or cell-cell adhesion. Both "homotypic" adhesion between neutrophils and "heterotypic" adhesion of neutrophils to the walls of blood vessels are required for cells to make their way out of the circulatory system and to cause inflammation.

Therapeutic concentrations of salicylates and NSAIDs, however, inhibit the cell-cell adhesion of human neutrophils. Furthermore, similar concentrations of sodium salicylate and aspirin have the same effect on neutrophils, even though they have widely divergent effects on prostaglandin synthase. It is therefore likely that the anti-inflammatory effect is related to the ability of both compounds to inhibit homotypic and heterotypic adhesion in neutrophils rather than to their unequal effect on prostaglandin synthesis.

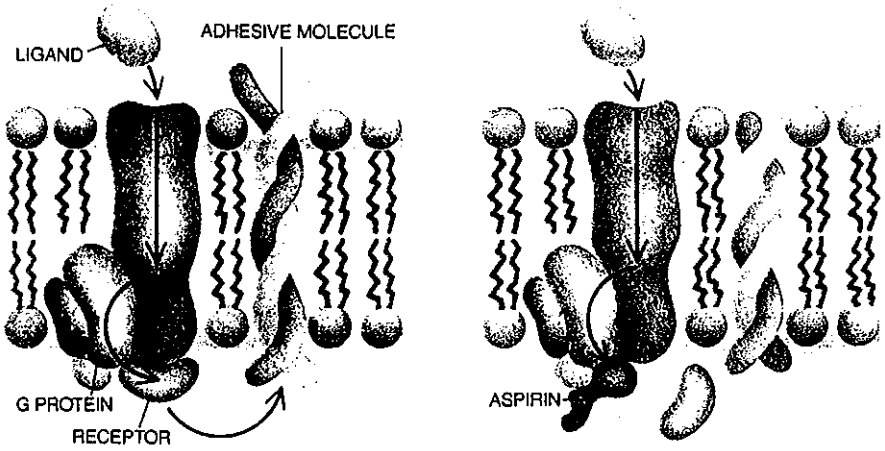
Inhibitory effects of NSAIDs on neutrophil activation can also be demonstrated in the clinic. The function of neutrophils derived from individuals given therapeutic doses of indomethacin, piroxicam or ibuprofen is significantly reduced. Neutrophils from the synovial fluid in the joints of patients with rheumatoid arthritis produced less superoxide anion—derivatives of molecular oxygen that can damage cells—after 10 days of therapy with piroxicam. And cells from normal volunteers given ibuprofen or piroxicam for three days failed to aggregate normally in response to chemoattractants.

All NSAIDs inhibit the homotypic adhesion of neutrophils, but they differ in their effects on other functions of the neutrophil. Piroxicam inhibits generation of superoxide anion by neutrophils exposed to various chemoattractants, but ibuprofen does not. Similarly, piroxicam and indomethacin inhibit the generation of superoxide anion by preparations of disrupted cells, but sodium salicylate, ibuprofen and the NSAID meclofenamate do not.

Vane's hypothesis is further weakened by findings from many laboratories, including mine, that the stable prostaglandins, E_1, E_2 and I_2, possess anti-inflammatory properties as well as proinflammatory ones. Robert B. Zurier of the University of Pennsylvania School of Medicine and others have shown that high doses of stable prostaglandins inhibit inflammation in animals with arthritis, and much lower doses inhibit similar inflammation induced by local skin irritants. It has also been clear since the early 1970s that PGI_2 and prostaglandins of the E type inhibit the activation in vitro not only of platelets but also of cells involved in inflammation, such as neutrophils and mononuclear phagocytes.

Paradoxically, both NSAIDs and prostaglandins of the E series similarly inhibit effects on the activation of neutrophils or platelets. Adding piroxicam to human neutrophils exposed to a chemoattractant can reduce superoxide anion generation by about 40 percent. Yet adding PGE_1 or PGE_2 at nanomolar to micromolar concentrations does not override the inhibition caused by piroxicam, as would be expected from the prostaglandins' proinflammatory capabilities. Instead it reduces superoxide anion generation by another 40 percent. More recent studies with the clinically useful PGE_1 derivative misoprostol also show synergistic rather than antagonistic effects between NSAIDs and prostaglandins.

Moreover, NSAIDs and prostaglandins have similar effects on the generation of such second messengers as calcium and cyclic adenosine monophosphate (cAMP), both of which transmit signals within cells. Increases in intracellular calcium induced by chemoattractants in human neutrophils are diminished not only by indomethacin but also by pretreatment of the cell with PGE_2. Chemoattractants increase levels of cAMP in the neutrophil slightly in order to provoke their effect. But prosta-



CELL MEMBRANES are the site of action for aspirin effects that do not depend on prostaglandin inhibition. The drug blocks transmission of chemical signals through the membranes by binding to a key regulatory protein. This prevents the first step in inflammation: adhesion of white blood cells to vessel walls.

glandins raise intracellular levels of cAMP by a larger amount, an effect that antagonizes cell activation. NSAIDs also enhance the increases of cAMP provoked by chemoattractants.

Some of the effect of NSAIDs on cells arises from interference with the binding of chemoattractants and other stimuli. These drugs inhibit the binding of at least some of these ligands with their receptors in the cell membrane, whereas acetaminophen, which fails to inhibit cell aggregation, has no effect on ligand binding.

The effects of NSAIDs on the binding of chemoattractants, however, are insufficient to explain their effects on neutrophils. NSAIDs inhibit cell activation in response to ligands whose binding they do not affect, such as $C5_a$ (a chemoattractant peptide), platelet-activating factor and leukotriene B_4. NSAIDs also inhibit activation in response to other stimuli. H. Daniel Perez of the University of California at San Francisco has shown that meclofenamate, for example, inhibits $C5_a$-induced neutrophil functions, without inhibiting binding of radiolabeled $C5_a$.

Because NSAIDs are acid, lipophilic molecules, they would be expected to alter membrane processes that depend on the overall mobility of membrane lipids. Salicylates at concentrations as low as 100 micromoles per liter decrease the viscosity of neutrophil membranes, whereas piroxicam and indomethacin increase viscosity at concentrations of 10 and 50 micromoles per liter, respectively. Acetaminophen, the analgesic, affects neither membrane viscosity nor the passing of chemical signals across the membrane.

Studies of purified membrane preparations and intact neutrophils show that NSAIDs interfere in particular with signals that depend on so-called G proteins for transduction through cell membranes. The evidence for this hypothesis begins with experiments that expose cells to pertussis toxin. This bacterial toxin interferes with signal transduction in a variety of cells, including the neutrophil, by altering certain G proteins in the plasma membrane. Neutrophils treated with toxin produce less superoxide anion when later exposed to chemoattractants.

Sodium salicylate similarly inhibits superoxide production—although far more modestly. Cells incubated with both pertussis toxin and sodium salicylate, however, regain their toxin-inhibited ability to generate superoxide anion. This paradoxical effect of salicylate suggests that salicylates interfere with the action of pertussis toxin near the site of its interaction with the G protein; they too must interact with the G protein in the cell membrane.

In addition, NSAIDs such as salicylate, piroxicam and indomethacin block the pertussis toxin-induced alteration of the G protein in purified neutrophil membranes. And salicylates and piroxicam inhibit in part other pertussis toxin-sensitive activities that follow cell activation.

All these NSAID effects have nothing to do with prostaglandin synthesis. A final blow to the generality of the prostaglandin hypothesis comes from one of the most primitive and ancient creatures, the marine sponge *Microconia prolifera*. This sponge, whose ancestry stretches back more than a billion years, offers a unique model for investigating the anti-inflammatory effects of NSAIDs.

The activation of sponge cells in the course of their aggregation is not influenced by stable prostaglandins, nor do the cells contain enzymes that could synthesize prostaglandins. Nevertheless, NSAIDs (but not acetaminophen) inhibit the aggregation of these cells just as they do that of neutrophils. Dispersed cells aggregate in response to a species-specific molecule weighing about 20 million daltons, called MAF. NSAIDs inhibit cell aggregation in response to MAF at concentrations similar to those that inhibit neutrophil aggregation. Because marine sponges cannot make prostaglandin, it is clear that these effects of NSAIDs—like those on insects, plants or human cells (neutrophils)—are unlikely to result from their inhibition of prostaglandin synthesis.

Vane's prostaglandin hypothesis explains a good part of the action of aspirin and related drugs, but much remains to be learned about the biology of these compounds as they interact with crucial systems of the cell. Nevertheless, it is reassuring to know that they have already helped to unravel biochemical pathways shared by creatures from which humans have been separated by a billion years of evolution.

FURTHER READING

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